The Effect of Soygurt Fortification with Black Rice Bran Extract Anthocyanin in Hyperlipidemia: Preliminary Step of Food Product Development

Enny Purwati Nurlaili
Faculty of Agricultural Technology
University of 17 Agustus 1945
Semarang, Indonesia
enny.purwati@gmail.com

Abstract

The main causes of hyperlipidemia include the consumption of a high-fat diet, which results in atherosclerosis and heart disease. Prevention of hyperlipidemia can be done by consuming foods rich in antioxidants, such as anthocyanins contained in black rice bran. This study aims to evaluate the anti-hyperlipidemic effect on soy fortified with black rice bran extract on humans, which in research conducted on Wistar *(Rattus norvegicus)* rat research animals. This study aims to evaluate the effect of anti hyperlipidemia on soygurt enriched with black rice bran extract on Wistar *(Rattus norvegicus)* rats. This research was conducted *in vivo*. Research materials included soygurt and black rice bran extract. The research stages included preparation of animal lab, 28 male Wistar rats, 3 weeks old; feed AIN-93G (G) and AIN-93M (M); adaptation a week; depletion period for 5 weeks, until hyperlipidemia with total cholesterol levels ± 200 mg/ml and total triglycerides ≥ 150 mg/dl. Separated into 4 diet treatment groups, namely without a standard diet, M diet + demineralized water (P = placebo); M diet + soygurt dried (K = control), M diet + 50 mg black rice bran extract enriched in soygurt (S-50), and M diet + 100 mg black rice bran extract enriched in soygurt (S-100) for 5 weeks. Taken blood from retroorbital flexus, euthanization, and surgery. Analyses were changed in total cholesterol levels, total triglyceride levels. Statistical analysis was performed by analysis of one-way variants (One Way Anova) with Minitab 17 at a significance level of 5%. If there are significant differences, then proceed with the Tukey's test ($p \leq 0.05$). The results showed that the S-100 group was able to reduce total cholesterol and triglyceride levels after the end of treatment at 95.13 mg/dl and 69.55 mg/dl. Changes in total cholesterol levels during the study, namely at the early until the induction increased by 57.19% and the induction to the end decreased by -48.19% had a positive effect on the treatment of S-50 and S-100 reducing total cholesterol levels. Whereas for the total triglyceride levels during the study, at the early until the induction increased by 46.51% and the induction to the end decreased by -48.42%, a positive effect of the treatment of S-50 and S-100 decreased total triglyceride levels.

Keywords
Soygurt, Fortification, Black Rice Bran Extract, Anthocyanin, Hyperlipidemia

1. Introduction

Hyperlipidemia is a disorder of lipid metabolism characterized by increased levels of cholesterol, LDL *(Low-Density Lipoprotein)* and decreased HDL (High-Density Lipoprotein). Factors triggering such conditions include a family history of dyslipidemia, hyperlipidemia, overweight, lack of physical activity, alcohol consumption, smoking, and diabetes. Besides a diet that is high in cholesterol such as cow brain, egg yolks, seafood, and red meat also can trigger the onset of hyperlipidemia (Price and Wilson 2006).

Research conducted by the Agency for Health Research and Development, in 2013, stated that there are people in Indonesia over the age of 15 years had higher levels of total cholesterol, triglycerides, and LDL above normal, and 60.3% of them had levels of LDL *near-optimal* and *borderline* high (100-159 mg/dl) (Agency for Health Research and Development 2013). Some researches suggest that there is a strong correlation between high LDL levels with the formation of atherosclerosis (Almatsier 2004).

One effective way to overcome hyperlipidemia by consuming foods that contain lots of antioxidants because its content can neutralize free radicals that cause disease caused by conditions such as hyperlipidemia, atherosclerosis (Ocean 2008).

Black rice has the content of antioxidants, especially anthocyanin, amounting to 10.70 ± 0.03 mg/g (Kong and Lee 2010). Black rice bran contains phenols, high anthocyanin. Brown rice and black rice diet can improve blood antioxidant levels and reduce the formation of atherosclerotic plaques in rabbit's hyperlipidemia (Ling *et al*. 2002). A Brazilian study showed that administration of a diet containing black rice cultivar IAC600 can control the lipidemia condition in rats (Salgado *et al*. 2010).

Soy milk is processed soy beverages containing high nutritional value, wherein the protein content reaches 3.5-4.0%. Besides the price is cheaper than cow's milk, soy milk also has other advantages, such as cholesterol-free and lactose-free, so consumed by people who are allergic to cow's milk (lactose intolerance). But people generally prefer cow's milk because of their unpleasant odors (beany flavor) in soy milk. One way to minimize these unpleasant odors is further processing into yogurt, known as soygurt. The culture used in the manufacture of soygurt in this study are Lactobacillus bulgaricus and Streptococcus thermophilus.

The steps in product development involves**:** Idea generation & screening, market research, product specification, feasibility study, process development, prototyping development and testing and launching**.** This paper is a part of product specification which is part of the overall development of food products.

2. Methods

2.1. Materials and Methods Research

The materials used were black rice that milled and taken black rice bran. The chemicals used included: HCl, acetic acid, the mice feed AIN-93G and AIN-93M (American Institute of Nutrition) (Reeves 1993). Kits for the determination of cholesterol and triglyceride levels, deionized water. For this study used several types of equipment, among others were, glass tools, analytical scales, oven, water bath shaker, muffle furnace, UV-VIS spectrometer, vortex, centrifuge (4 ºC), rotary evaporator, freeze dryer. The animal lab used were male Wistar rats types aged 3 weeks.

2.2. The Course of Study

Before making the feed for the animal, a preparation to extract rice bran black by way of bran of black rice is extracted with a solution of acetic acid 3% in demineralized water, shaker at 40 °C for 60 minutes and then stored at 4 °C for 24 hours was carried out. Then do the filtering, in a rotary evaporator for 4 hours at 40 °C and in the freeze dryer. Manufacture soygurt using bacteria L. bulgaricus and S. thermophilus. Furthermore, making the feed treatment conducted under standard feed AIN-93G and AIN-93M.

Before treatment, the animal lab first underwent a period of adaptation for 7 days and was fed at libitum. At the beginning of the treatment performed on the analysis of mouse blood plasma for total cholesterol and triglyceride levels, to determine the condition of hyperlipidemia or not. Rats that have undergone hyperlipidemia was then divided into 4 groups @ 7 tail, and maintained for 5 weeks in individual cages, with the provision of the basal diet and drink deionized water ad libitum. During this period, they were given soygurt drinks enriched with anthocyanin extracts of black rice bran as force-feeding. Analysis of cholesterol and triglyceride levels was carried out at the beginning, during and at the end period of the study of the 28 tails carried division of the group into four dietary treatment groups, i.e. without a standard diet, a diet M + demineralised water (P = placebo); diet M + soygurt dried (K = control), M + soygurt dried enriched rice bran black extract levels of 50 mg (S-50), and a standard feed AIN-93M + soygurt dried enriched rice bran black extract levels of 100 mg (S-100) for 5 weeks. Blood sampling performed by retro-orbital flexus and then do analysis.

Data were analyzed by analysis of variance single-lane (One Way Anova) with Minitab 17 at a significance level of 5%. If there was a significant difference, then followed by Tukey's test.

3. Result and Discussion

3.1. Development Rat Weight

The development of body weight every week during the study can be seen in Figure 1.

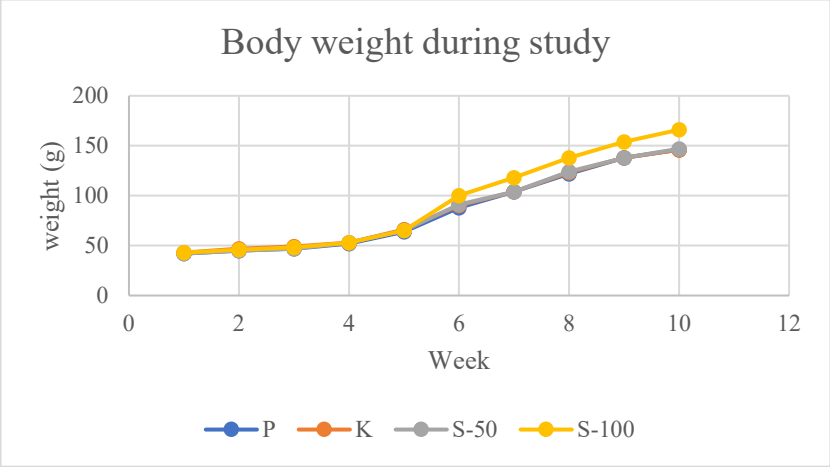

Figure 1. The development of body weight every week.

The development of rat body weight every week during the study through week 10 (Figure 1), showing an increase in weight every week in all groups of rats, significantly occur after the maintenance of up to 8 weeks.

Based on statistical analysis with ANOVA method RAL against the growth of body weight of rats showed no significant difference ($p \leq 0.05$). Thus, we can say that diet and long maintenance treatment did not significantly affect the growth of body weight of rats. Rats that were fed with food containing S-100 experienced higher growth than then ones treated with standard food. And there was a tendency of the consumer to have the highest growth. This suggests that the protein contained in soygurt played a role in the growth of rats. Zhao (2004), earning rats fed a diet of food containing soybean products that have a weight higher than the growth casein diet.

3.2. Plasma Cholesterol Levels

The development of cholesterol levels during the study, until the 10th week during the study period is shown in Figure 2.

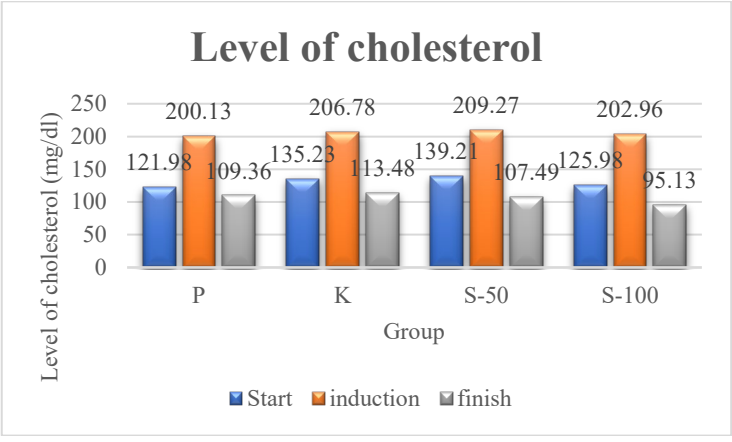

Figure 2. Levels of cholesterol at the start, induction, and end of the study.

The intervention of diet with enriched feed to the treatment of black rice bran extract anthocyanin on soygurt effect on cholesterol levels of mice hyperlipidemia, characterized by a decrease ranging from 95.13 to 113.48 mg/dl. It is evident from Figure 2, which shows that at the end of the study mice group S-100 experienced a significant reduction in cholesterol levels compared to other groups. The conditions show that anthocyanin contained in the extract significantly help to decrease cholesterol levels in rats hyperlipidemia. The results are consistent with the results of the study stating that granting diet with black rice aleuronic anthocyanin containing 5 g/100 g of feed given for 16 weeks, was able to inhibit the formation of atherosclerosis lesion rats deficient in apolipoprotein. Wounds that are not inhibited will lead to the accumulation of cholesterol and plaque formation. Besides it reduces oxidative stress and inflammation (Xia *et al*. 2003). Reduction of plaque formation was also examined by Ling et al. (2002) which are on the outer layer of black rice diet as much as 5 g/100 g of feed given to the rabbits that have been given a high cholesterol diet for 2 months.

3.3. Changes in Plasma Cholesterol Levels

Changes in plasma cholesterol levels can be seen in Figure 3.

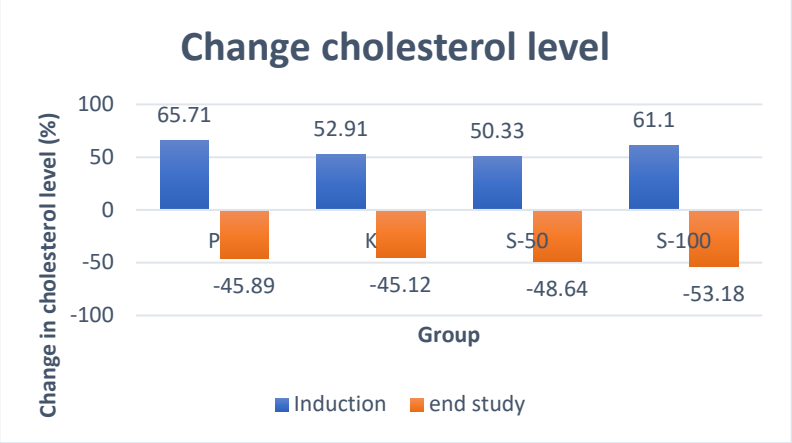

Figure 3. Changes in cholesterol levels at baseline, induction and at the end of the study.

Percentage changes in plasma cholesterol levels showed that the levels increase at the beginning until the induction of 50.33 to 65.71%. While the induction treatment fitter diet decreased levels of 48.64 to 53.13%. This indicates that the induction treatment of S-50 and S-100 can lower cholesterol levels compared to other treatments.

3.4. Plasma Triglycerides

Overview of the development of triglyceride levels is presented in Figure 4. Figure 4 shows that at the end of the study the S-100 experienced a significant decrease in triglyceride levels compared to other groups. Treatment enrichment anthocyanin black rice bran extract on soygurt effect on rat triglyceride levels hyperlipidemia, characterized by the decline, total triglyceride levels with levels ranging from 69.55 to 88 mg/dl. Triglyceride levels associated with LDL in the human body.

Triglycerides enter the blood plasma in two forms namely chylomicron formed from intestinal absorption after eating fatty foods, and hepatic VLDL formed with the help of insulin. Triglycerides outside the liver will be hydrolyzed by the enzyme lipoprotein lipase. The rest will be metabolized into LDL (Simatupang 1997).

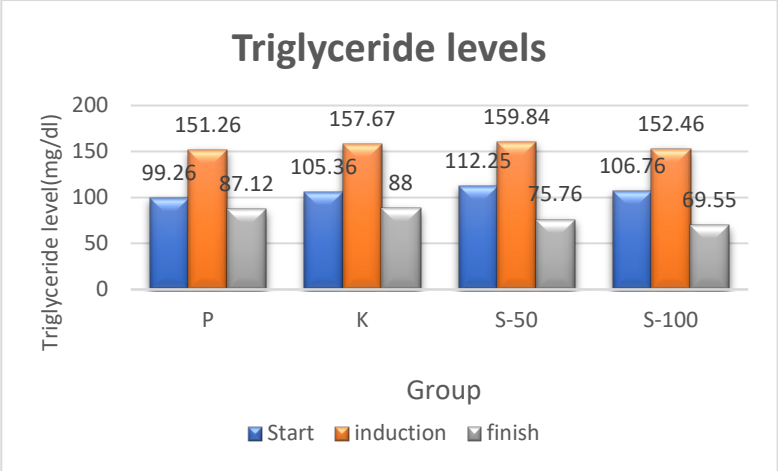

Figure 4. Triglyceride levels at baseline, induction and at the end of the study.

3.5. Changes in Plasma Triglyceride Levels

Changes in plasma cholesterol levels can be seen in Figure 5.

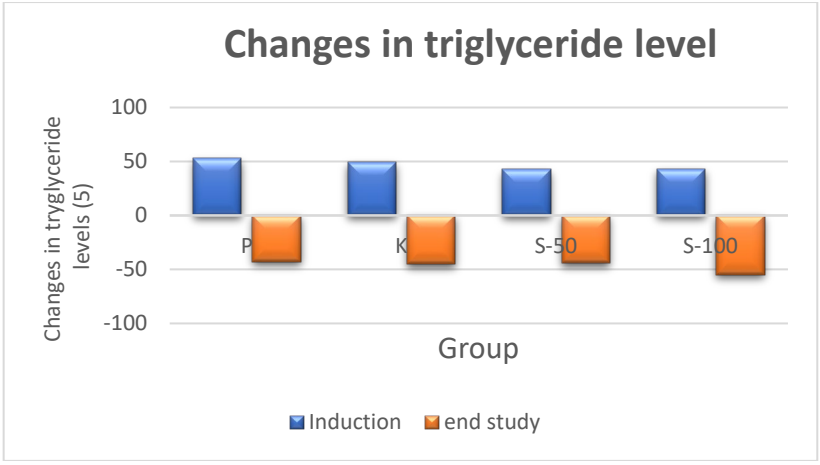

Figure 5. Changes in triglyceride levels at baseline, induction and at the end of the study.

The percentage change in plasma triglyceride levels showed that levels increase at the start until the induction of 42.40 to 52.39%. While the induction treatment fitter diet decreased levels of 42.40 to 54.38%. This indicates that the induction treatment of S-50 and S-100 can lower triglyceride levels compared to other treatments.

Hyperlipidemia (high cholesterol and triglycerides) caused by the consumption of fat, can also be caused due to the consumption of carbohydrates. Digestion of carbohydrates to produce monosaccharides glucose main will be stored in the liver in the form of glycogen. To produce energy, glycogen is broken down into glucose and subsequently produce pyruvate and acetyl-CoA through glycolysis and eventually form CO_2, H_2O, and ATP. If ATP is not used, then the acetyl-CoA would not enter the citric acid cycle but will be used to form the fatty acid through esterification with glycerol and result in triglycerides (Zakir 2005). Triglycerides enter the blood plasma in two forms namely chylomicron formed from intestinal absorption after eating fatty foods, and hepatic VLDL formed with the help of insulin. Triglycerides outside the liver will be hydrolyzed by the enzyme lipoprotein lipase. The rest will be metabolized into LDL (Simatupang 1997). LDL excessive hyperlipidemia conditions can cause constriction of blood vessels through the formation of plaque on the walls of blood vessels. LDL oxidation by free radicals of oxygen causes

LDL easily sticks to the walls of blood vessels and cause of plaque and can cause endothelial dysfunction (Diaz *et al*. 1997). Antioxidants are known to prevent the oxidation of LDL and increase HDL. The results of this study indicate the effect of hyperlipidemia in soybean enriched with rice bran extract can reduce cholesterol and triglyceride levels to a certain degree.

4. Conclusion

The results showed that group S-100 can lower total cholesterol and triglycerides after the end of treatment, respectively amounted to 95.13 mg/dl and 69.55 mg/dl. Changes in total cholesterol levels during the study in the beginning until the induction rose by 57.19% and induction to the end fell by -48.19% positive effect of the treatment of S-50 and S-100 lowers total cholesterol levels. As for the total triglyceride levels during the study in the beginning until the induction rose by 46.51% and induction to the end fell by -48.42% positive effect of the treatment of S-50 and S-100 lowering the total triglyceride levels. Decreasing cholesterol and triglyceride levels that can be produced from strengthening soybean with black rice bran is expected to improve human health at work. The results of this laboratory research, then can be tested on humans and increased to the next stage in the development of food products.

References

Agency for Health Research and Development, *Basic Health Research Riskesdas 2013*, Ministry of Health, Jakarta, 2013.

Almatsier, S., *Basic Principles of Nutrition*, PT Gramedia Pustaka Utama, Jakarta, 2004.

Diaz, M. N, Frei, B, Vita, J. A, and Keaney, J. F., Antioxidants and Atherosclerotic Heart Disease, *The New England Journal of Medicine: Research and Review*, vol. 337, no. 6, pp. 408-416, 1997.

Kong, S. and Lee, J., Antioxidants in milling fractions of black rice cultivars, *Food Chemistry*, vol. 120, no.1, pp. 278-281, 2010.

Ling, W. H., Wang, L. L., and Ma, J., Supplementation of the black rice outer layer fraction to rabbits decreases atherosclerotic plaque formation and increases antioxidant status, *The Journal of Nutrition*, vol. 132, no. 1, pp. 20-26, 2002.

Ocean, J., Free radicals in erythrocytes and leukocytes, *Clinica Chimica Acta*, vol. 390, no. 1-2, pp. 1-11, 2008.

Price, S. A. and Wilson, L. M., *The Pathophysiology of Concept Clinical Disease Processes*, Publisher EGC medical books, Jakarta, 2006.

Reeves, P. G., Nielsen, F. H., and Fahey, G. C., AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent die, *The Journal of Nutrition*, vol. 123, no. 11, pp. 1939-1951, 1993.

Salgado, J. M., de Oliveira, A. G. C., Mansi, D. N., Donado-Pestana, C. M., Bastos, C. R., and Marcondes, F. K., The role of black rice (*Oryza sativa L.*) in the control of hypercholesterolemia in rats, *Journal of Medicinal Food*, vol. 13, no. 6, pp. 1355-1362, 2010.

Simatupang, A., *Cholesterol, Hypercholesterolemia and The Drugs Against IT - A Review, Review of Literature*, Mirror World of Medicine, Jakarta, 1997.

Xia, M., Ling, W. H., Ma, J., Kitts, D. D., and Zawistowski, J., Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in Apolipoprotein E deficient mice, *The Journal of Nutrition*, vol. 133, no. 3, pp. 744-751, 2003.

Zakir, S., Effect of poparo glucoce in reducing blood levels in Diabetics, *Thesis*, Institute of Animal Nutrition and Feed Technology, Faculty of Animal Husbandry, University of Agriculture Fasalabad, Pakistan, 2005.

Zhao, C., Giusti, M. M., Malik, M., Moyer, M. P., and Magnuson, B. A., Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth, *Journal of Agricultural and Food Chemistry,* vol. 52, no. 20, pp. 6122-6128, 2004.

